Original Article

Impact of feed characteristics for monitoring AFB1 contamination in commercial concentrate feeds used in small holder dairy farms, Chiangmai, Thailand

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Abstract

The objective of this study was to determine the impact of abnormal physical characteristics for monitoring aflatoxin B1 (AFB1) contamination in commercial dairy concentrate feeds. The study was conducted during March to May 2013 using 82 dairy farms in Chiang Mai province, Thailand. All feed samples were collected from new packages and their physical characteristics were subsequently determined. AFB1 concentrations in the samples were measured using the enzyme-linked immunosorbent assay (ELISA), and feed samples with AFB1 of more than 5 μg/Kg were determined as AFB1 contaminated samples. The impact of physical characteristics on AFB1 contamination were evaluated using a multiple logistic model with a p-value less than 0.05. Forty percent (39 of 97) of the feed samples were contaminated with AFB1. Concentrate feeds with rancid or sour odors had a higher risk of AFB1 contamination (OR =4.79, P=0.03), as did samples with a high appearance of a brightly illuminated fluorescence on feed after testing with black light (OR= 24.04, P = 0.0002). Farmers can use these indicators as screening tests before purchasing the feed.

Keywords: aflatoxin B1; concentrates; contamination; dairy cow; physical characteristics, black light

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Introduction

Aflatoxin M1 (AFM1), a carcinogenic mycotoxin in dairy products, is a serious concern as the consumption of these products poses health risks, particularly to young people (Pradini et al., 2009). In Thailand, many reports have shown high levels of AFM1 in both raw milk and drinking milk (Suriyasathaporn & Nakprasert, 2012; Chaisri et al., 2017). The main reason for AFM1 contamination is the consumption of feed contaminated with AFB1 (Bantaokul & Ruangwises, 2010). Not only causing AFM1 contamination in milk, AFB1 is also the most toxic mycotoxin metabolite causing a reduction of feed consumption, growth retardation, reduced performance, immune suppression, irritated tissues, causing abortions and death (Hall and Wild, 1994). Because of the potential hazards of this toxin, many countries have set guidelines of acceptable levels in animal feed to reduce the risk of AFM1 contamination in milk. The regulation in Europe and the United states limits AFB1 in animal feed to 5 and 20 µg/Kg (European Food Safety Authority, 2004), while the regulation in Thailand limits total aflatoxin to 100 µg/Kg and 200 µg/Kg for young and mature animals, respectively (Thai Ministry of Agriculture and Cooperative regulation, 2016).

In Thailand, most farmers feed their cows with a classical mixed ration of roughage and concentrates (Mongkon et al., 2014; Oberheu & Dabbert, 2011). For convenience, many dairy farmers purchase commercial concentrate feeds (Mongkon et al., 2014; Oberheu & Dabbert, 2011; Mongkon et al., 2017; Suriyasathaporn & Nakprasert, 2012). Regardless of farm management factors, especially storage practices, related to AFM1 contamination (Mongkon et al., 2014; Suriyasathaporn & Nakprasert, 2012), the easiest way of preventing AFM1 contamination in milk is to purchase feed with minimal AFB1 contamination. Abnormal characteristics of commercial concentrate feeds might be used to monitor AFB1 contamination. Therefore, the objective of this study was to determine the impact of feed characteristics for monitoring AFB1 contamination in commercial dairy concentrate feeds.

Materials and Methods

Chemicals and Reagents All reagents were of analytical grade. Methyl alcohol was purchased from Merck (Darmstadt, Germany). DOA-Aflatoxin ELISA test kit (Department of Agriculture, Ministry of Agriculture Cooperatives, Thailand) was purchased from Higher Enterprises Co.Ltd, Pathum Thani, Thailand.

Study design and feed collection A cross-sectional study was performed during March to May 2013 using all 82 smallholder dairy farms from a dairy cooperative in Chiang Mai province, Thailand. To minimize factors relating to farm management on AFB1 contamination of commercial dairy concentrate feed, all samples were collected from new packages of all concentrate feeds using in farms. One kilogram of each sample was collected, kept on ice in a plastic zip lock bag, transported to the laboratory and stored in a freezer (-18°C) until analysis.

Physical feed characteristics The physical characteristics of feed samples were established by the same investigator, including proportions of cracked particles, presence of abnormal odor, presence of abnormal color, proportion of pack particle and proportion of bright fluorescence after testing with black light. Based on a normal pellet length of 2 cm, cracked particles were defined as a pellet length ≤1 cm by visual observation. Samples were categorized into three levels: not cracked (less than 10% cracked particles); partially cracked (10 to 50% cracked particles); and most cracked (more than 50% cracked particles). Sour or rancid odors were defined as abnormal odor of the feeds. The normal color of dairy concentrate feed is yellow or light brown, dependent on the raw materials. Darker than normal, dark brown or black were considered abnormal colors. The pack particle was the packed characteristic of the feed sample after hand squeezing. The procedure for fluorescence light testing for feed samples has been described by Mongkon et al. (2014); a positive result was defined as a brightly illuminated fluorescence appearing on the pellet area. Samples were categorized as negative, partly positive and highly positive to black light tests when the percentages of positive pellets were 0%, 0-10%, or ≥10% of all pellets, respectively.

Aflatoxin B1 analysis Each feed sample was carefully mixed and finely ground, and then extracted by putting 20 g of the ground sample into an Erlenmeyer flask to which 100 ml of 70% methyl alcohol was added. The flask was shaken at 300 rounds per min for 30 mins, after which the mixture was filtered by Whatman no. 4 paper. The AFB1 level was measured by DOA-Aflatoxin ELISA test kit (Postharvest and Processing Research and Development Division, Thailand). The kit had a recovery between 82-100 % and a sensitivity limit of 0.4 µg/Kg (Chinaphuti et al., 2002). The analysis was performed according to the kit instruction. Briefly, 50 µl of either AFB1 standard (0, 0.2, 0.5, 1, 2 ng/ml) or the diluted samples were added into antibody coated wells; then 50 µl of Aflatoxin B1-Horseradish Peroxidase (AFB1-HRP) conjugate was added to each well, slightly shaken and incubated at room temperature for 30 mins. The contents of the well were dumped into the appropriate waste container and the plate was washed 3 to 5 times by 0.01M phosphate buffer saline with 0.5% Tween 20 (PBS-T). Tetramethylbenzidine substrate (100 µl) was added to each well and incubated for 10 mins at room temperature. The reaction was stopped by adding 100 µl of 0.3M phosphoric acid. The solutions were read at 450 nm using an automated MicroELISA spectrophotometer reader. The results of absorbance were expressed as percentages of maximal binding as follows:

% maximal binding = B/B0 x 100

Where B= mean absorbance of feed sample and

B0= mean absorbance of AFB1 standard at 0 ng/ml

The standard curve was generated by plotting the concentration of AFB1 with % maximal binding in Log base 10 values as shown in Fig 1. To test the recovery of the DOA-Aflatoxin ELISA method based on the

standard curve, concentrate feed samples were spiked with AFB1 at a concentration of 50 µg/Kg, selected as the half concentration of a previous recovery test study (Chinaphuti et al., 2002).

Statistical analysis Data was described as frequencies for categorical variables. Feed samples with AFB1 at concentrations higher than 5 µg/kg, the regulatory limit for dairy animal feed of the European Food Safety Authority (EFSA) (European Food Safety Authority, 2002), were determined as samples contaminated with AFB1. For univariable analysis, the impact of feed characteristics on AFB1 contamination were separately determined using Fisher's exact chi-square test. The final model of impacts of feed characteristics on AFB1 contamination was determined using a multiple logistic model with a backward selection method (SAS Institute, 1997). All factors were first entered and subsequently remained in the model when their P-values, indicated by the likelihood ratio tests, were less than 0.10.

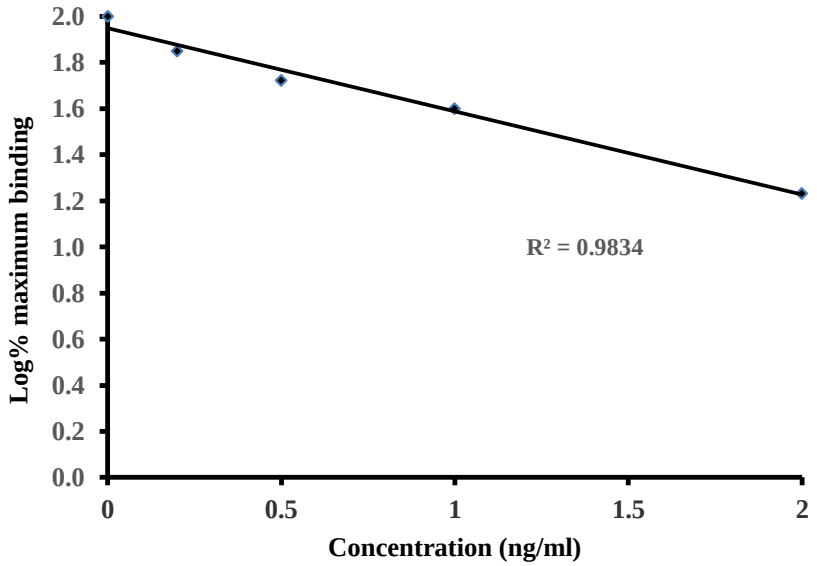

Figure 1 The enzyme-linked immunosorbant assay (ELISA) standard curve for the determination of AFB1 concentration

Results

The accuracy of AFB1 measurement was measured by the ELISA standard curve (Fig. 1). The correlation coefficient (R^2) at 0.98 indicated a good validity of the AFB1 measurement. The test was repeated five times and the recovery rate was 88.4%.

The mean ± SD of AFB1 contamination of all 97 feed samples from 82 farms, in which 15 farms used 2 concentrate feeds and 67 farms had only 1 concentrate feed, was 5.41 ± 3.61 µg/kg, ranging from 1.10 to 23.61 µg/Kg. Forty percent of the samples (n = 39) exceeded the EFSA allowable limit, and were considered contaminated with AFB1. The characteristics of the commercial concentrates are shown in Table 1. The commercial concentrates exhibited high levels of abnormal or improper characteristics including abnormal color 12.4% (12/97), sour or rancid odor 19.6% (19/97), abnormal pack particle 27.8% (27/97), abnormal cracked particles 43.3% (42/97) and positive to black light test 44.3% (43/97). From univariable analysis, 3 out of 5 tested characteristics were significantly associated with AFB1 contamination (P<0.01) including cracked particles, abnormal odor and positive results having bright illumination under black light test. The highest percentages of AFB1 contamination (44.3%) were found in samples with the most cracked particles (93.8%), highly positive under black light test (87%) and sour or rancid odors (73.7%).

From the final analysis, the factors having impact on AFB1 contamination included abnormal odor and having a brightly illuminated light under black light test (Table 2). The concentrates with a sour or rancid odor had a higher risk of AFB1 contamination than those with a normal odor (OR = 4.79, P = 0.03). Feed samples with more than10% of brightly lit area or highly positive had a higher risk of AFB1 contamination than samples without any bright area (OR = 24.04, P = 0.0002).

Discussion

In the last decade, many countries in tropical regions have encountered aflatoxin contamination problems. In the present study, we found approximately 40% of feed samples were contaminated with AFB1. The high incidence of AFB1 contamination found in this study was in line with previous studies in other tropical countries (Arunvipas et al., 2009; Meemark & Sakdinun, 2006). Meemark & Sakdinun (2006) found that aflatoxin B1 was detected in 287 feed samples (78.42%) ranging from 0.40-23.97 µg/Kg. Likewise, in this study, AFB1 concentrations found in concentrated feed ranged between 1.105 to 23.61 µg/Kg; these results were consistent with ranges previously reported in Thailand (Charoenpornsook & Kavisarasai, 2014). Due to the uses of ELISA, instead of the uses of high performance liquid chromatography as a standard method for aflatoxin measurement, aflatoxin contamination parameter, defined at AFB1>5

µg/Kg, were used instead of their AFB1 concentrations.

In this study, abnormal commercial concentrate characteristics were found in high percentages, ranging between 12.4% (12/97) to 44.3% (43/97), as shown in Table 1. In general, dairy concentrate feed pellet characteristics, including length or particle size, depend on the manufacturing process (Bryden, 2012; Gizachew et al., 2016). After production, feed pellets are stored in plastic bags to prevent humidity and fungal growth (Martinez & Woloshuk, 2012; Moreira et al., 1996). Among the abnormal characteristics examined in this study, cracked or damaged feed pellets were the only factors caused by a physical mechanism (Dorner et al., 1989; Gizachew et al., 2016). The other characteristics we examined required biological processes. Fungi and mycotoxin contamination, including AFB1, could occur in the raw materials before manufacturing (Das et al., 1993; Gizachew et al., 2016).

Table 1 Association of feed characteristics of concentrate feed samples (n = 97) with AFB1 contamination (indicated by an AFB1 concentration above 5 µg/kg, the allowable contamination limit in complete or concentrate feed for dairy cattle as defined by EFSA, 2002)

Feed characteristic	Total (n = 97)	AFB1 contaminated sample (n = 39)		P-value
		Number	Percentage (%)	
Cracked particle				<0.001
Mostly cracked	16	15	93.8	
Partially crack	26	9	34.7	
Not cracked	55	15	27.3	
Odor				0.0014
Sour or rancid	19	14	73.7	
Normal	78	25	32.1	
Color				0.2142
Abnormal	12	7	58.3	
Normal	85	32	37.7	
Pack particle				0.3609
Abnormal	27	13	48.2	
Normal	70	26	37.1	
Black light test				<0.001
Mostly positive	23	20	87	
Partially positive	20	8	40	
Negative	54	11	20.4	

Table 2 The final model indicating physical feed characteristics associated with AFB1 contamination in commercial dairy concentrate feed

Feed characteristic	Estimate	SE[a]	P-value	Odds ratio	95% CI[b]
Odor					
Sour or rancid	1.57	0.73	0.03	4.79	1.15 – 19.94
Normal	----------Reference----------				
Black light test					
Partially positive	1.15	0.66	0.08	3.17	0.85 – 11.77
Mostly positive	3.18	0.85	0.0002	24.04	4.54 – 127.18
Negative	----------Reference----------				

[a]SE, Standard error
[b]95% CI, 95% Confidence interval

From simple analyses, cracked particles, a sour or rancid odor, and a positive black light test significantly increased the likelihood of AFB1 contamination (Table 1). However, the cracked particle variable was not included in the final model of this study (Table 2), in contrast to our previous study that included cracked particles (as well as a positive black light test) as a risk factor for AFM1 contamination in milk (Mongkon et al., 2017). The luminescence or positive black light result indicated the presence of fungi, especially AFB1-producing fungi (Moreira et al., 1996), and was related to aflatoxin contamination of corn kernels (Abbas et al., 2004; Adams et al., 1993). Grain or feed spoiled by fungal growth had an abnormal odor, including musty, sour or putrid smell (Atanda et al., 2016; Miller, 1995). Humidity in cracked feed pellet particles, either during manufacturing or later storage, might promote fungal growth and AFB1 production (Atanda et al., 2016; Das et al., 1993). In addition, cracked pellets are more likely to be contaminated with fungi (Adams et al., 1993).

In conclusion, the physical feed characteristics especially abnormal odors and a positive black light test, were associated with AFB1

contamination in dairy concentrate feeds, and can be used as factors for monitoring AFB1 contaminated feeds especially before purchase by farmers.

Acknowledgements

The authors thank the dairy farmers of the Patung Dairy Cooperative, Chiang Mai for their participation and appreciate the Faculty of Veterinary Medicine, Chiang Mai University for supporting.

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บทคัดย่อ

ผลของลักษณะอาหารเพื่อเฝ้าระวังการปนเปื้อนอะฟลาทอกซินบี 1 ในอาหารข้นที่มีจำหน่ายที่ใช้ในฟาร์มโคนมรายย่อยในเชียงใหม่ ประเทศไทย

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วัตถุประสงค์ของการศึกษานี้เพื่อหาผลกระทบของลักษณะทางกายภาพที่ผิดปกติเพื่อช่วยในการตรวจสอบการปนเปื้อนอะฟลาทอกซินบี 1 (AFB1) ในอาหารสำเร็จรูปโคนม ดำเนินการศึกษาระหว่างเดือนมีนาคมถึงพฤษภาคม พ.ศ. 2557 โดยใช้ฟาร์มโคนมในจังหวัดเชียงใหม่จำนวน 82 ฟาร์ม เก็บตัวอย่างอาหารและสังเกตลักษณะทางกายภาพของอาหารหลังจากเปิดถุงอาหารทันที วัดปริมาณอะฟลาทอกซินบี 1 โดยใช้วิธี Enzyme-linked immunosorbent assay (ELISA) และตัวอย่างอาหารที่มีอะฟลาท็อกซินเกิน 5 ไมโครกรัม/กิโลกรับถูกกำหนดเป็นตัวอย่างที่มีการปนเปื้อน AFB1 ทดสอบความสัมพันธ์ระหว่างลักษณะทางกายภาพของอาหารและการปนเปื้อนอะฟลาทอกซินโดยใช้ multiple logistic model และกำหนดค่า p-value เท่ากับ 0.05 พบว่าตัวอย่างอาหารร้อยละ 40 (39 จาก 97 ตัวอย่าง) ปนเปื้อนอะฟลาทอกซินบี 1 อาหารข้นที่มีกลิ่นเหม็นหืนหรือเหม็นเปรี้ยวเสี่ยงต่อการปนเปื้อนอะฟลาทอกซินมากกว่าอาหารที่มีกลิ่นปกติ (Odds ratio (OR)=4.79, P=0.03) เช่นเดียวกับตัวอย่างอาหารที่เรืองแสงฟลูออเรสเซนต์หลังจากทดสอบด้วยหลอด black light (OR=24.04, P=0.002) เกษตรกรผู้เลี้ยงโคนมสามารถใช้ลักษณะดังกล่าวเพื่อคัดกรองอาหารก่อนซื้อเข้าฟาร์มได้

คำสำคัญ: อะฟลาทอกซินบี 1 อาหารข้น การปนเปื้อน โคนม ลักษณะทางกายภาพ แบล็คไลต์

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